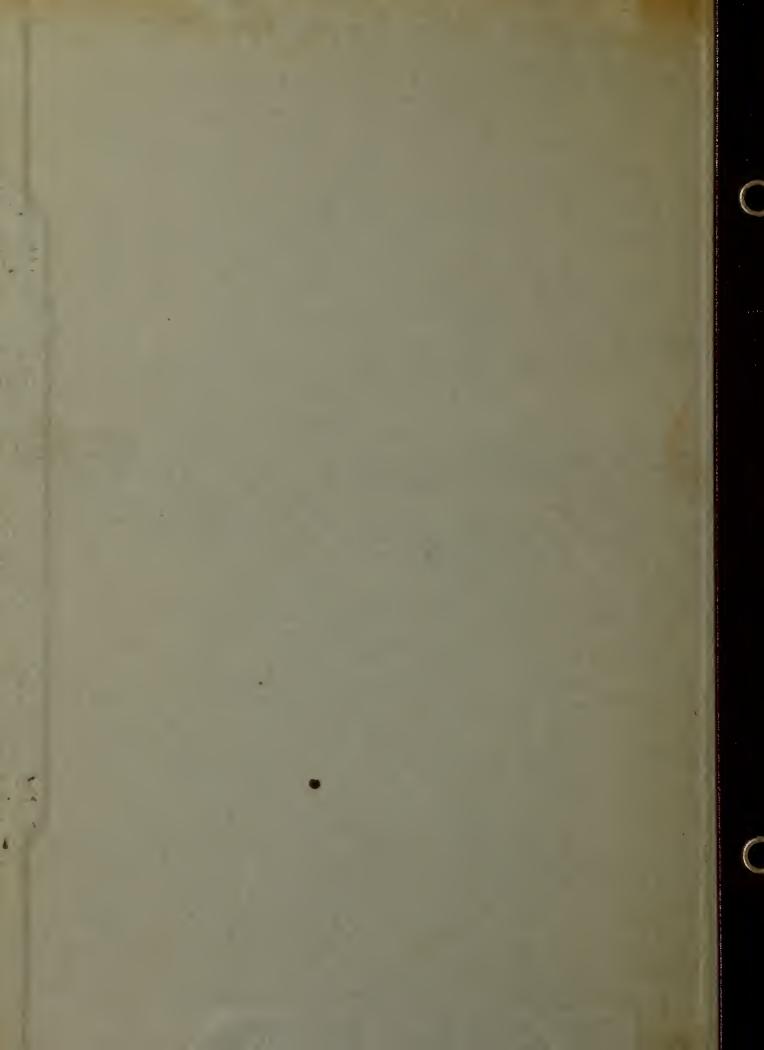
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### BOSTON UNIVERSITY GRADUATE SCHOOL

Thesis

THE HISTOLOGY OF THE STEM BARK OF RHAMNUS
PURSHIANA VARIETY ANONAEFOLIA

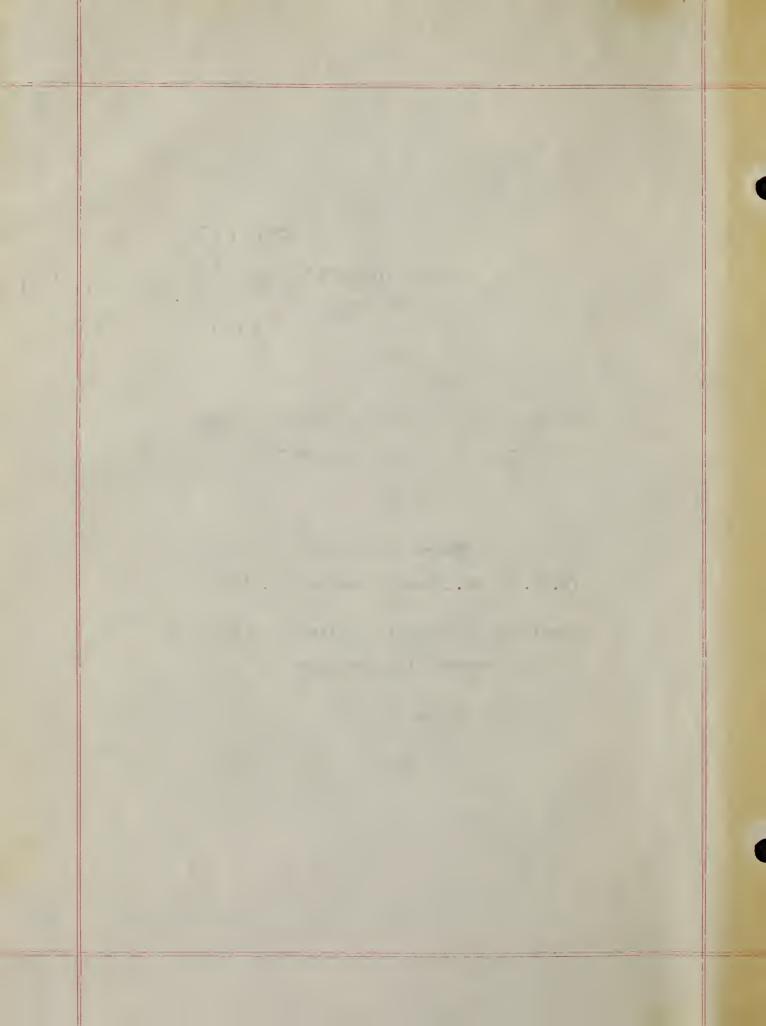
by

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submitted in partial fulfilment of the requirements for the degree of

Master of Arts

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#### Introduction

The writer became interested in this problem because of the fact that the stem bark of the pure species of Rhamnus

Purshiana De Candolle is extensively used in medicine, and, although the variety anonaefolia (Greene) Jepson also grows in the state of California, a thorough search of the literature shows that no one has ever studied the histological features of the stem bark of the variety to determine similarities and differences between the stem barks of the pure species and its variety.

Originally Greene gave the variety full specific rank, and later Jepson reduced it to a variety of Rhamnus Purshiana DC. There still seems to be a question in the minds of some taxonomists (especially Dr. Carl Wolf of the Rancho Santa Ana Botanic Gardens, who has just published a monograph on the genus Rhamnus in California) as to whether or not it is the same as the pure species. Therefore, the writer thought an histological study of the stem bark might be of some value and interest in this connection.

## <u>Purshiana</u> variety anonaefolia

E. L. Greene, Pittonia, iii. 16(1906), first described this plant and gave it full specific rank. His description

follows:

"Rhamnus anonaefolia. Deciduous shrub with few and not slender leafy and puberulent branchlets: thin and not strongly veiny leaves very ample, 3 to 5 inches long, many 2 1/2 inches wide above the middle, all of distinctly obovoid outline and more or less cuneate-tapering below the middle, or even from towards the apex, retuse or obtuse, or some even distinctly obcordate, finely serrulate, especially above the middle, the petioles 1/2 to 1 inch long: the solitary few-fruited umbel on a peduncle about as long as the petiole; fruit large 3 seeded.

Known only from the mountains of Placer County,
California, where it was collected in 1892 by Mr. A. M.
Carpenter. It was distributed under the name of R. Purshiana,
and is related to that species; but the remarkable cuneate
cut of the leaves, being very constant forbids its being referred to that species; which does not connect with it geographically."

W. L. Jepson, Manual of Flowering Plants of California 614(1925), gives the plant the rank of a variety and his description follows:

"R. purshiana DC. Cascara Sagrada. Small tree or shrub 8 to 20 feet high; leaves in a tuft at end of branch-lets, thinnish, deciduous, elliptic-oblong, obtuse or slightly

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cordate at base, obtuse or abruptly blunt-pointed at apex, serrulate, 2 1/2 to 5 (or 8) in. long; petioles tomentulous; flowers 5-merous; berry black, with 3 (rarely 2) nutlets. - Mountain slopes and canons, near Mendocino and Humboldt Cos. near the coast; n. to Washington. Var. anonaefolia Jepson n. comb. Leaves obovate or oblong, the larger 4 to 7 in. long and 1 1/4 to 2 1/2 in. wide, mostly cuneately tapering at base. - Bear Valley, Nevada Co., to Mount Shasta. (R. anonaefolia Greene).

#### Method of Procedure

Stem barks of varying ages and thickness from the youngest to that most fully matured were studied in transverse section, and the oldest bark was studied in radial-longitudinal and tangential-longitudinal sections. The normal-butyl alcohol method as outlined by Dr. Conrad Zirkle of Harvard University was used in preparing many of the sections. In other instances the barks were boiled in water to soften the tissues and sections cut directly on a sliding microtome. Permanent mounts of many of the sections were made and in preparing these the stains safranin and methyl green were used. Considerable difficulty was encountered in preparing perfect sections because of the large amount of stereome tissue present in the bark. For this reason, it seemed better to make representative drawings of the sections and then having these drawings

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complete descriptions are included for all sections studied and photographed drawings of representative sections are included.

A resume of the similarities and chief differences between the mature stem barks of the pure species Rhamnus Purshiana DC. and its variety anonaefolia (Greene) Jepson is included in the Summary and Conclusion of the Thesis.

Two new and interesting facts pertaining to the histological features of the bark of the genus Rhamnus are also included in this discourse.

Many sections were studied in various chemical reagents to determine the nature of the cell walls and cell-contents. Following is a record of various tests employed by the writer in determining the nature of the cell walls and cell-contents.

Concentrated sulphuric acid and solution of Soudan Red (0.1%) in glycerin-alcohol was used to determine the suberization of cork cells, etc.

A 1% solution of phloroglucin in 95% alcohol and concentrated hydrochloric acid was used to determine the presence or absence of lignin in fibers, stone cells, etc.

Ammonium hydroxide T.S., and potassium hydroxide T.S.

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were used in testing for the presence of anthraquinone derivatives.

Iodine water (saturated aqueous solution) was used in testing for the presence of starch in the cells of the cortex, phloem and medullary rays.

Schulze's Maceration Process was used in isolating stereome elements. This consisted of subjecting small pieces of the bark to the action of a mixture of 50 cc. of concentrated nitric acid and 1 Gram of potassium chlorate, and heating until red vapors cease to be evolved and then washing the residue that remains undissolved in cold distilled water.

Corallin-soda solution was employed in studying the sieve elements.

Measurements of various tissues and cells was made with a standardized ocular micrometer, the values for each space on the ocular micrometer being 15.00 microns with the 16 mm. objective and 3.52 microns for each space on the ocular micrometer with the 4 mm. objective. These standardizations were made with a tube length of 160 mm., and a 10x ocular.

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#### Sources of Material and Correspondence

Considerable correspondence took place with the following men in the state of California, the object of this correspondence being to obtain authentic material for the thesis and to obtain their opinion as to the value of the thesis.

Mr. Woodbridge Metcalf, Extension Forester, University of California, College of Agriculture, Berkeley, California.

Mr. Garth F. Flint, Junior Forester, Tahoe National Forest, Nevada City, California.

Mr. Henry A. Kloppenburg, District Ranger, U.S.D.A., Plumas National Forest, Quincy, California.

Dr. Carl Wolf, Director, Rancho Santa Ana Botanic Garden, Anaheim, Orange County, California.

The material for the thesis was shipped to me through the courtesy of Mr. Garth F. Flint and Mr. Henry A. Kloppenburg mentioned above. The shipments of stem bark and whole stems also included leaves from the plants and these were compared with herbarium material at the Massachusetts College of Pharmacy, Boston, and the Gray Herbarium, Cambridge, Mass. The material compared favorably and was determined to be authentic by the writer.

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# Histological Descriptions of Stem Barks of Various Ages

Transverse Section of 1 year old Stem Bark (Plate I)

Diameter of whole stem 1.5 mm., and average thickness of bark of stem 0.375 mm.

#### Cork

One or more layers of uniformly thin-walled cork cells, on the outer surface of which may be seen the remains of epidermis in the process of sloughing off. The walls of the cork cells are suberized and the cork cells are filled with a yellowish amorphous substance.

### Phellogen or Cork Cambium

A layer of very thin meristematic cells in a rapid state of subdivision producing phellem on the outer surface and phelloderm on the inner surface.

#### Phelloderm or Secondary Cortex

A layer or two of slightly elongated, somewhat collenchymatic cells frequently containing chloroplasts.

### Primary Cortex

A few layers of rounded, thin-walled parenchyma cells containing chloroplasts, starch in the form of minute grains, and a few monoclinic prisms (rhombohedra) and rosette

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aggregates of calcium oxalate are present in some of the cells.
Rifts appear in various parts of this region and are quite
conspicuous just outside the pericycle.

#### Pericycle

One or usually two layers of sclerenchyma fibers which appear rounded to polygonal in shape when observed singly. These fibers have thick, non-lignified lamellated walls. This layer of fibers is not continuous, but is interrupted here and there.

#### Phloem

A narrow region of phloem cells and sieve tubes. The medullary rays are mostly one cell wide and due to dark-colored contents are quite conspicuous; they vary from straight to slightly curved in their outer portion. An occasional monoclinic prism (rhombohedron) or rosette aggregate of calcium oxalate may be noted in this region. A small group of sclerenchyma fibers surrounded by crystal fibers containing rhombohedra of calcium oxalate was noted in the inner part of the phloem of one series of sections examined, but this is not typical of young bark during its first year of growth.

A DESCRIPTION OF THE PARTY OF T AND THE RESIDENCE OF THE PARTY THE RESERVE OF THE PARTY AND ADDRESS OF THE PA . The state of the THE RESERVE OF THE PARTY OF THE and the first specific of the state of the s Transverse Section of 2 to 3 year old Stem Bark (Plate II)

Diameter of whole stem averagely about 3 mm., and average
thickness of bark 0.450 mm. to 0.600 mm.

#### Cork

Six to eight layers of uniformly thin-walled cork cells with suberized walls, the dead outer layers of which are filled with air, the inner living layers are filled with a yellowish amorphous substance.

#### Phellogen

Consisting of a layer of rapidly dividing thin-walled cells, giving rise to phellem on its outer surface and phelloderm on its inner surface.

### Phelloderm

consisting of two to three layers of somewhat thick-walled cells containing many chloroplasts. An occasional monoclinic prism (rhombohedron) or rosette aggregate of calcium oxalate may be noted in some of these cells.

### Primary Cortex

Eight to ten rows of more or less rounded to slightly elongated parenchyma cells. These cells contain chloroplasts, a few small starch grains, monoclinic prisms (rhombohedra) of calcium oxalate which are quite conspicuous, and rosette aggregates of calcium oxalate which are extremely abundant.

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The sclerenchyma content of the primary cortex and pericycle regions varies considerably. In some sections the pericycle is evident and shows non-lignified sclerenchyma fibers which occur singly or in groups, while in other sections these fibers are absent. In each entire section there is usually noted two to three groups of strongly lignified stone cells, or in some cases the stone cells may occur singly; the groups of stone cells are partially surrounded by crystal cells containing rhombohedra of calcium oxalate. In still other sections one may find a few groups of lignified sclerenchyma fibers surrounded by crystal fibers containing rhombohedra of calcium oxalate but these are rare. Rifts occur here and there throughout the cortex.

#### Phloem

A fairly broad area consisting of numerous phloem patches separated by medullary rays one to two cells thick. The phloem is composed of small rounded to slightly wavy—walled phloem parenchyma cells and rounded sieve tubes. The companion cells are difficult to see in this type of section. Some of the phloem parenchyma cells contain rosette aggregates or monoclinic prisms (rhombohedra) of calcium oxalate, the rosette form being more common. Some of the phloem parenchyma cells contain small starch grains, and the medullary rays contain starch in the form of small grains. Sclerenchyma

. . The Total Control of the Control o  elements, when present, are in the form of strongly lignified stone cells, the walls of which are sometimes lamellated and of varying thickness. The groups of stone cells are surrounded by crystal parenchyma containing rhombohedra of calcium oxalate. Occasionally a few small groups of lignified sclerenchyma fibers surrounded by crystal fibers containing rhombohedra of calcium oxalate may be observed.

- I adam a constant and all the constant and a cons . The second sec Transverse Section of 5 year old Stem Bark

Diameter of entire stem 12 mm., and average thickness of the bark was 0.750 to 0.850 mm.

#### Cork

Five or six layers of typical phellem cells with thin, uniformly thickened, suberized walls and containing a yellowish-brown amorphous substance.

#### Phellogen

A layer of meristematic cells in a rapid state of subdivision, producing phellem on its outer surface and phelloderm on its inner surface.

#### Phelloderm

Two to three layers of cells somewhat elongated, with thickened walls and containing many chloroplasts. Occastonally a rosette aggregate or rhombohedral crystal of calcium oxalate may be seen in a few of these cells.

### Primary Cortex

A fairly broad area of slightly elongated to rounded parenchyma cells containing a few small starch grains in some of the cells. Rosette aggregates of calcium oxalate and monoclinic prisms (rhombohedra) of calcium oxalate also occur in the parenchyma cells of the cortex, the rosette form being the more common, however. Strongly lignified stone cells

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occur singly, but more commonly in groups which are partially surrounded or sometimes completely surrounded by crystal parenchyma containing rhombohedra of calcium oxalate; these groups of stone cells are generally present in the inner portion of the cortex and occur in groups. The individual fibers noted here and there are non-lignified to lignified (slightly) and have thick lamellated walls.

#### Phloem

A relatively broad area consisting of patches of phloem parenchyma and sieve tubes separated by medullary rays which are one to three but usually two cells in width. Strongly lignified stone cells occur singly or in groups scattered throughout the protophloem region, the thickness of their walls varying considerably and some of their walls are slightly lamellated. These groups of stone cells are sometimes partially surrounded by crystal parenchyma containing rhombohedra of calcium oxalate. A few groups of strongly lignified sclerenchyma fibers are seen here and there in the region of the metaphloem. The sclerenchyma (bast) fibers are surrounded by crystal fibers containing rhombohedra of calcium oxalate. The sclerenchyma fibers, surrounded by crystal fibers, are not as uniformly arranged in tier-like fashion as in the older stem bark. The companion cells are difficult to distinguish from the sieve tubes in this type of section.

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<u>Transverse Section of 8 to 10 year old Stem Bark</u>

<u>Diameter of entire stem 20 mm., and average thickness</u>

<u>of the bark was 1.250 mm. to 1.5 mm.</u>

#### Cork

Six to eight layers of typical cork cells with thin, uniformly thickened, suberized walls and containing a yellow-ish amorphous substance. Occasionally fragments of lichen tissue are present on the outer layers of cork cells. The cells of this region are arranged in regular rows radially.

#### Phellogen

A layer of meristematic cells in a rapid state of subdivision, producing phellem on its outer surface and phelloderm on its inner surface.

#### Phelloderm

Three or four layers of somewhat elongated, thick-walled cells rich in chloroplasts.

### Primary Cortex

A fairly broad area of rounded to slightly elongated parenchyma cells. These cells contain starch in the form of small rounded grains, monoclinic prisms (rhombohedra) of calcium oxalate, or more commonly rosette aggregates of calcium oxalate. Scattered throughout this area are large groups of stone cells or single stone cells, the shape of the

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individual stone cells varying considerably as well as the thickness of their walls and the degree of lignification. Some of the stone cells are non-lignified, others slightly lignified and still others lignified to a considerable degree. The groups of stone cells are partially to more or less completely surrounded by crystal cells containing rhombohedra of calcium oxalate. Occasionally groups of sclerenchyma fibers occur in this region and usually they are distinguished by having thick, non-lignified lamellated walls and a small lumen.

## Phloem

A broad area composed of phloem patches separated by medullary rays one to three cells in width. The medullary rays have a straighter course than in the more mature bark. Rounded to slightly irregular-shaped phloem cells and sieve tubes comprise a large part of this region. The companion cells of the sieve tubes are difficult to observe in this type of section. Starch in the form of small spherical grains is found in some of the phloem parenchyma cells and, to a relatively greater extent, in the medullary ray cells. Stone cells occur singly or in groups in the outer phloem region and are thick-walled, strongly lignified and partially surrounded by crystal cells containing rhombohedra of calcium oxalate. Some of the stone cells have lamellated walls.

Sclerenchyma (bast) fibers, surrounded by crystal fibers containing rhombohedra of calcium oxalate, are arranged in tierlike fashion throughout the phloem patches between the medullary rays, the individual fibers being rounded to chiefly angular and slightly lignified. Monoclinic prisms (rhombohedra) of calcium oxalate, or more commonly, rosette aggregates of calcium oxalate occur in many of the phloem patches. Sections treated with an alkaline solution do not give any color reaction, thus indicating the absence of anthraquinone derivatives in appreciable quantities.

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Transverse Section of Mature Stem Bark (Plate III)

The section described below was obtained from trunk bark,

the diameter of the trunk being 85 mm., and the thick
ness of the bark averagely 3 mm.

#### Cork

Consisting of ten to twenty layers of rectangularshaped cork cells with more or less uniformly thickened and suberized walls. The outer layers are usually dead and filled with air, and frequently show fungal hyphae and apothecia from epiphytic lichens; the inner layers are filled with a yellowish to orange-yellow amorphous substance. In one series of section, a group of stone cells was observed, this group of stone cells being completely surrounded by crystal cells containing rhombohedra of calcium oxalate and the whole mass in turn being completely enveloped by cork cells. While the phenomenon of stone cell and crystal formation in the phellem layers has been recorded previously, it is not recorded in the literature as having been observed in the genus Rhamnus. It is possible, of course, that the occurrence of the stone cells and crystals in this instance was due to some mechanical irritation suffered by the bark, since most of the sections did not exhibit this condition.

# Phellogen

A layer of meristematic cells in a rapid state of

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subdivision producing phellem on its outer surface and phelloderm on its inner surface.

## Phelloderm

containing chloroplasts. This tissue is developed, at most, in small quantity only, the cells are distinguished from the phellogen cells by having thicker walls, from the cork cells by not being suberized, and from the cells of the cortex (usually) by their arrangement in radial rows. Occasionally groups of stone cells and crystals (rhombohedra of calcium oxalate) are seen wedged into the inner layers of this region.

## Primary Cortex

A broad zone of rounded to slightly elongated, thin-walled parenchyma cells containing a few chloroplasts, monoclinic prisms (rhombohedra) of calcium oxalate and rosette aggregates of calcium oxalate. Scattered throughout the cortex are numerous groups of stone cells, varying from single stone cells to large, elongated groups with sclerenchyma fibers, crystal cells and crystal fibers adjacent to them or partially surrounding them. The stone cells and crystal fibers are strongly lignified. In the region of the pericycle isolated groups of sclerenchyma fibers and crystal fibers were observed. Some of the cortical cells contain a few small starch grains. Occasional rifts occur in the

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cortical area, but these are not conspicuous.

## Phloem

A very broad area, composed of phloem patches between conspicuous medullary rays. The medullary rays are one to three cells wide in this kind of section, mostly two cells wide. The medullary rays have granular contents and contain starch in the form of small rounded grains. The contents of the medullary ray cells do not give any reaction for anthraquinone derivatives with an alkaline solution as is characteristic of most members of the Rhamnaceae. Repeated tests for anthraquinones with ammonium hydroxide T.S., and potassium hydroxide T.S. failed to produce a characteristic pink or red coloration. The medullary rays are wavy, particularly in the protophloem region and there is a tendency toward dome-shaped convergence of the medullary rays in the protophloem. The phloem is composed of phloem parenchyma cells which are rounded to slightly irregular in shape; these cells contain a very small amount of starch in the form of rounded grains, and numerous rosette aggregates and rhombohedra of calcium oxalate. The starch grains are of the simple spheroidal type and have a maximum diameter of 7 The rhombohedra of calcium oxalate have a maximum length of 31.68 microns. The rosette aggregates of callium oxalate have a maximum diameter of 40.16 microns. The sieve tubes are numerous and look very much like the phloem

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parenchyma in this type of section. The companion cells are not clearly differentiated from the sieve tubes in this type of section.

In the protophloem stone cells occur either singly or in groups. The stone cells are strongly lignified and in some instances are partially surrounded by sclerenchyma fibers and crystal fibers. Numerous bast fibers, surrounded by crystal fibers, are arranged in tier-like fashion throughout the entire phloem area. Both the bast fibers and stone cells are strongly lignified. The bast fibers are rounded to polygonal and have slightly lamellated and greatly thickened walls with a narrow lumen in the center of each cell.

# Radial-longitudinal Section of Mature Bark (Plate IV)

An examinations of this type of section shows the medullary rays to be from ten to thirty cells in height. The stone cells were quite elongated in some instances and the thickness of the walls shows great variation with a corresponding variation in the size of the lumen; some of the stone cells had distinctly lamellated walls and in all cases were strongly lignified. The bast fibers are elongated, taperended, and have a narrow lumen, thick walls and frequently show prominent pore canals in their walls. The bast fibers are usually surrounded by conspicuous elongated crystal fibers containing rhombohedra of calcium oxalate. The sieve tubes

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are very characteristic, showing either straight or oblique end walls with plates on all walls and they have a distinctly beaded appearance in this type of section. Very little callus had developed on most of the plates observed. The companion cells are somewhat shorter than the sieve tubes and look like the phloem parenchyma cells in most instances and frequently their walls are beaded as in the case of the sieve tubes.

Tangential-longitudinal Section of Mature Stem Bark (Plate V)

In this type of section the medullary rays appear spindle-shaped and are usually one to three cells wide, occasionally up to four cells wide (not commonly as in the case of the stem bark of the pure species). The other elements of the phloem were very similar in appearance to that recorded under the radial-longitudinal section.

Study of Stereome Elements from Mature Stem Bark (Plate VI)

The thicker stem bark was subjected to Schulze's Maceration Process, i.e., digested in a mixture of 50 cc. of concentrated nitric acid and 1 Gram of Potassium Chlorate, and heated until red fumes were no longer evolved and then the residue washed in distilled water. This process

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separates and isolates sclerenchyma elements. These elements were studied very carefully and it was observed that the stone cells vary considerably in size and shape, being rounded, narrowly elongated, to very irregular in shape; their walls vary from non-lamellated to slightly or even distinctly lamellated in some instances and the walls vary from being thick or thin with a correspondingly narrow or broad lumen. The stone cells were strongly lignified and up to 119.68 microns in length.

The sclerenchyma elements in the form of fibers from the cortex and phloem regions were taper-ended, considerably elongated, thick-walled with a narrow lumen and pore canals were frequently observed in their walls. The sclerenchyma fibers attained a maximum length of 770 microns. They were strongly lignified and generally surrounded by crystal fibers containing monoclinic prisms (rhombohedra) of calcium oxalate.

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Summary of Histological Differences and Similarities

of the Stem Barks of Rhamnus Purshiana DC. and Rhamnus

Purshiana variety anonaefolia (Greene) Jepson

A survey of the literature on the histological features of the stem bark of Rhamnus Purshiana DC. shows that most of the work done in the past was on the thick, mature stem bark. Specific details in regard to the histological features of the stem bark of Rhamnus Purshiana DC. were obtained from the works of Greenish (4), Sayre (6), and Youngken (8) and serve as the basis for the comparisons with the histological features variety anonaefolia of the stem bark of Rhamnus Purshiana (Greene) Jepson. The reason that the mature bark of the pure species is the only one which has been studied is probably due to the fact that only mature stem bark is used medicinally. Therefore, the following comparisons will be between mature stem barks of the pure species and its variety.

The microscopical characteristics of the sclerenchyma fibers, crystal fibers, rosette aggregates of calcium oxalate, monoclinic prisms (rhombohedra) of calcium oxalate, sieve tubes, phloem parenchyma, starch, and cortical cells are practically identical in all respects in the stem barks of the two plants.

The most conspicuous differences noted by the writer

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#### were as follows:

- 1. The medullary rays of the variety give no reaction with an alkaline solution, indicating absence of anthraquinone derivatives in appreciable amounts. The medullary ray cells of the stem bark of the pure species are immediately colored pink or red when treated with an alkaline solution. The alkaline solutions employed were potassium hydroxide T.S., and ammonium hydroxide T.S. This fact is quite interesting because most members of the Rhamnaceae are characterized by the fact that they give the above color reaction with an alkaline solution.
- 2. The medullary rays, as observed in tangential—longitudinal sections, are up to four cells wide in both the pure species and variety, but in the pure species they are commonly up to four cells wide, whereas in the stem bark of the variety they are most commonly up to three cells wide and only occasionally up to four cells wide.
- 3. The maximum height of the medullary rays in the stem bark of the variety was thirty cells, whereas the maximum height recorded in the literature for the stem bark of the pure species was twenty-five cells high.
- 4. The stone cells in the stem bark of the variety are not as frequently lamellated as the stone cells in the

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stem bark of the pure species.

- 5. The medullary rays are not as distinctly convergent in the protophloem of the stem bark of the variety as in the protophloem of the stem bark of the pure species.
- 6. The cork layers of the stem bark of the variety sometimes show groups of stone cells surrounded by monoclinic prisms (rhombohedra) of calcium oxalate. This condition has never been recorded in the cork cells of the stem bark of the pure species.

## Conclusion

The results of this investigation show that it is possible to distinguish between the stem barks of Rhamnus Purshiana DC. and Rhamnus Purshiana variety anonaefolia (Greene) Jepson, by means of histological differences, micro-chemical reactions, and physical differences in the gross structure and appearance of the two barks.

The chief points of distinction are as follows:

- (a) The medullary ray cells of the stem bark of the variety give no micro-chemical reaction with alkaline solutions for the presence of anthraquinone derivatives, whereas the medullary ray cells of the stem park of the pure species give a positive test for the presence of anthraquinone derivatives.
- (b) The medullary rays in the stem bark of the variety are usually three cells wide, occasionally up to four cells wide, whereas in the stem bark of the pure species the medullary rays are commonly up to four cells wide.
- (c) The medullary rays are up to thirty cells in height in the stem bark of the variety, whereas the maximum height recorded for the medullary rays in the stem bark of the pure species is twenty-five cells high.

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- (d) The cork cells of the variety occasionally show groups of stone cells imbedded therein and surrounded by crystal cells containing rhombohedra of calcium oxalate, whereas this condition has never been observed in the cork cells of the stem bark of the pure species.
- (e) The maximum thickness of the stem bark of the variety is three millimeters, whereas the stem bark of the pure species attains a maximum thickness of five millimeters quite commonly.
- (f) The stem bark of the pure species is usually grayish-brown to dark brown in color, whereas the stem bark of the variety is always grayish. Both barks are frequently seen with numerous apothecia of lichens on their outer surface.
- (g) Although the stone cells in the stem barks of both plants have similar characteristics in regard to size, variation in shape and thickness of walls, and degree of lignification, the walls of the stone cells in the stem bark of the variety are not as frequently or as conspicuously lamellated as those in the stem bark of the pure species.

from the facts correlated above it is clearly evident that the barks of the two plants differ histologically

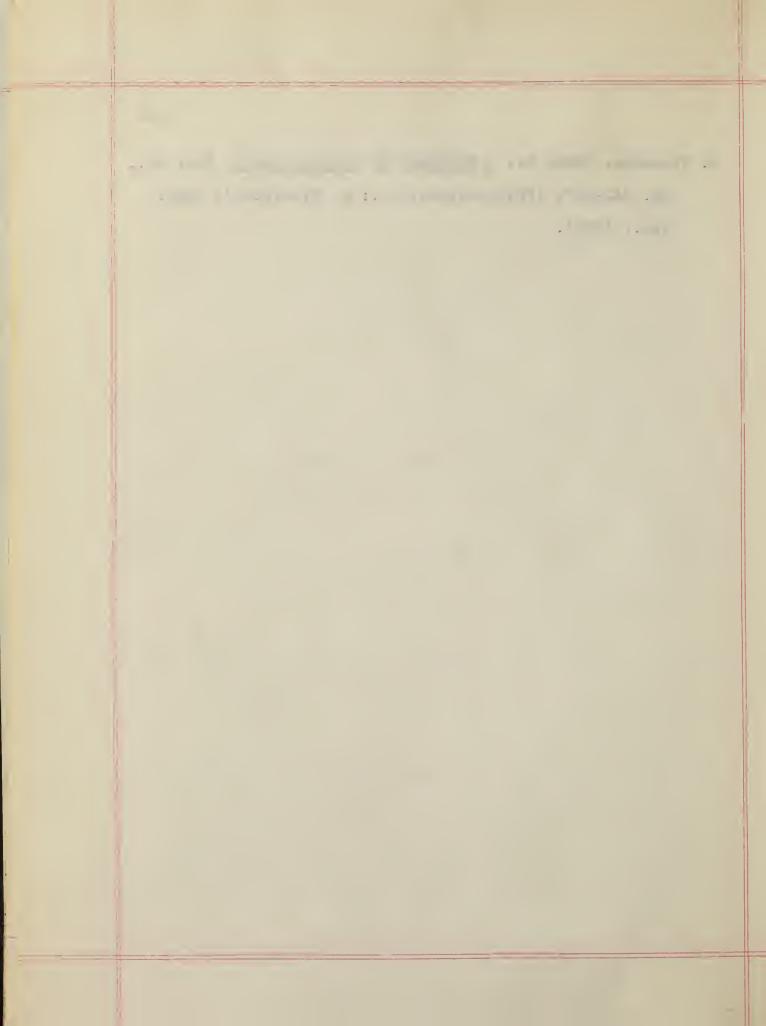
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in several respects and that there is justification in considering anonaefolia as a variety of the pure species, Rhamnus Purshiana DC., from the histological standpoint.

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P .

# List of abbreviations and their equivalent botanical terms used in labeling the following plates

bf	• • • • •	bast fibers
С	•••••	primary cortex
cr.f.	• • • • •	crystal fibers
k	• • • • •	cork (phellem)
kk	• • • • •	cork cambium (phellogen)
m.p.	•••••	monoclinic prisms (rhombohedra) of
		calcium oxalate
m.r.	• • • • •	medullary rays
p	• • • • •	phloem (phloem parenchyma)
p.f.	• • • • •	pericyclic fibers
ph	• • • • •	phelloderm (secondary cortex)
r	• • • • •	rift
r.cr.	•••••	rosette crystals of calcium oxalate
s	•••••	sieve tubes
scl	•••••	sclerenchyma fibers
st	• • • • •	stone cells

### Plate I

Transverse Section Stem Bark - 1 year old

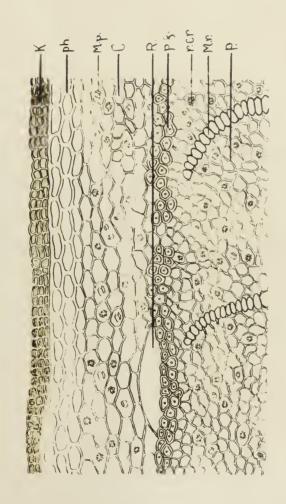
Average thickness of bark 0.375 mm.

Magnification of photographed drawing approximately 175x.

For description see pages 9-10



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### Plate II

Transverse Section Stem Bark - 2-3 year's old

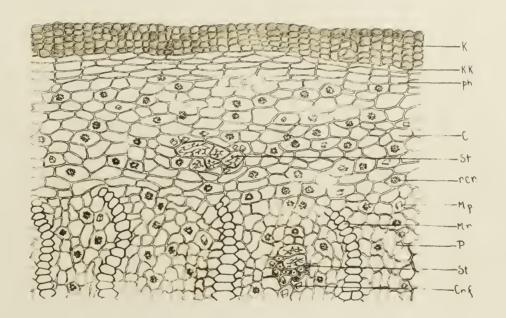
Average thickness of bark 0.525 mm.

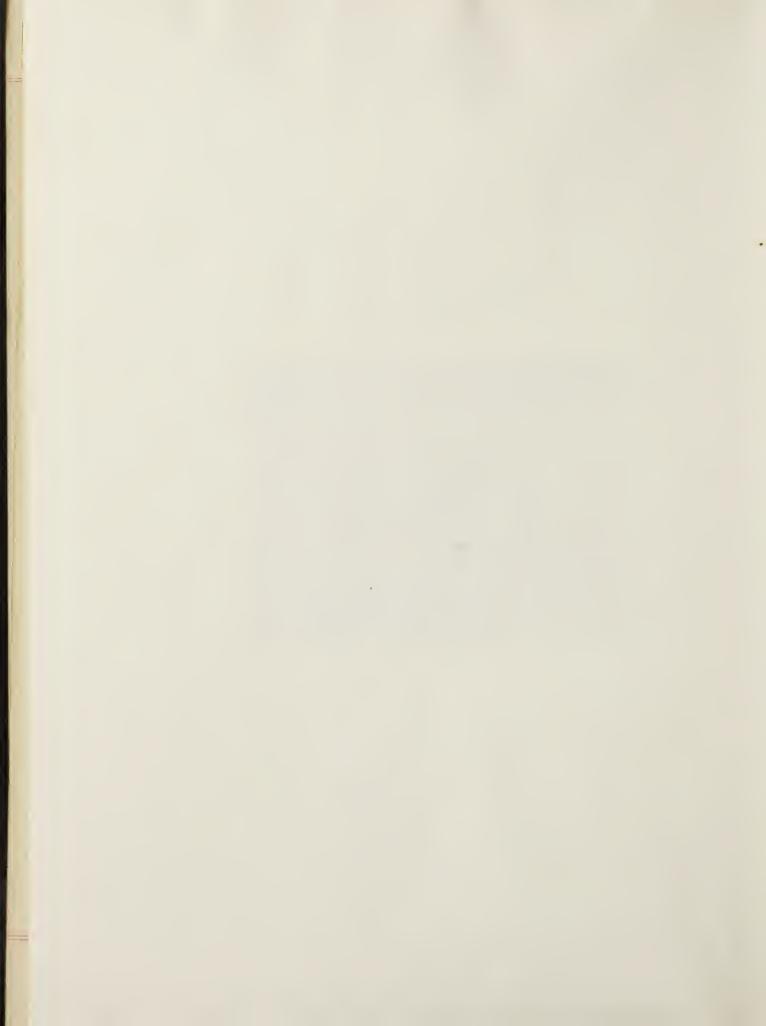
Magnification of photographed drawing approximately 140x.

For description see pages 11-13

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# Plate I





### Plate III

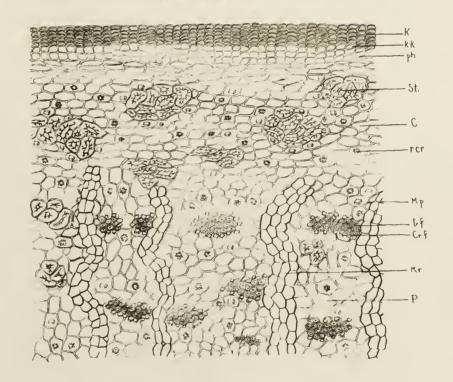
Transverse Section of Mature Stem Bark
Average thickness of bark 3 mm.

Magnification of photographed drawing approximately 50x.

For description see pages 19-22

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# Plate II



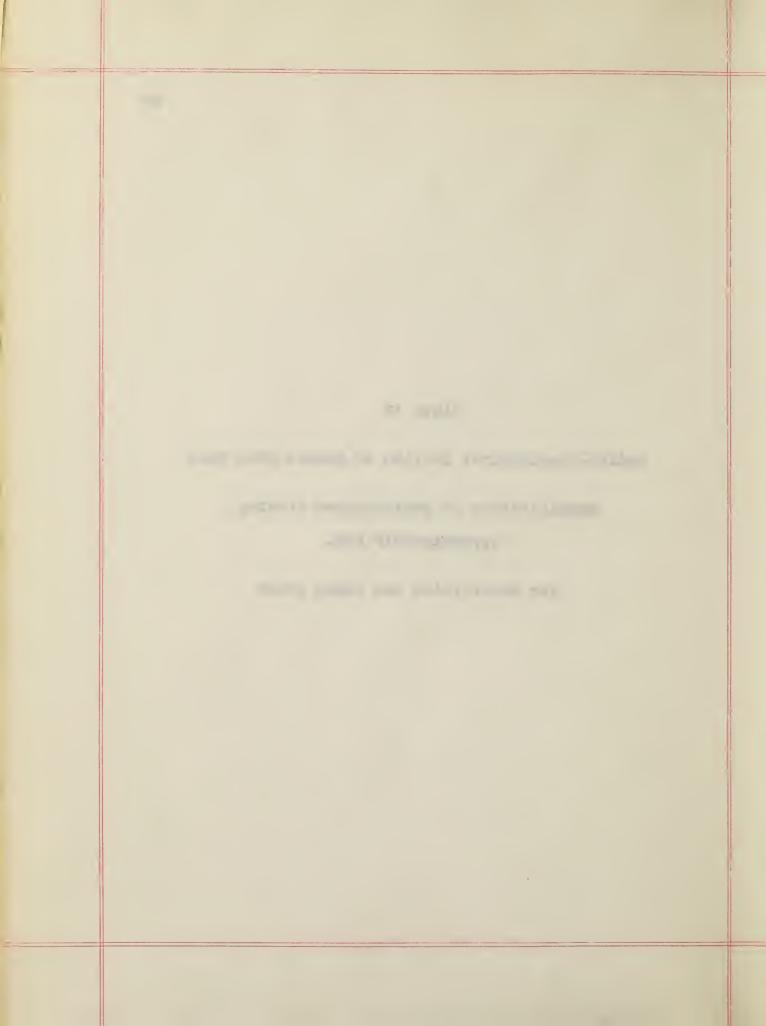


## Plate IV

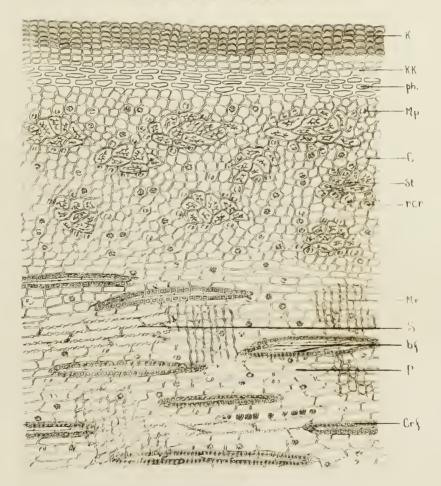
Radial-longitudinal Section of Mature Stem Bark

Magnification of photographed drawing approximately 60x.

For description see pages 22-23



# Plate I





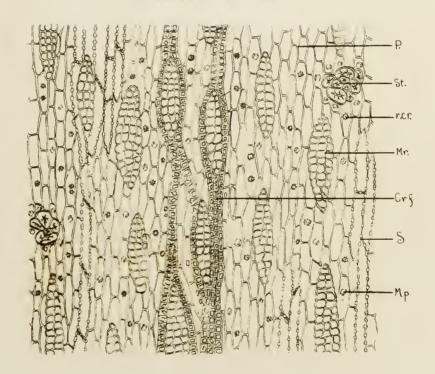
## Plate V

Tangential-longitudinal Section of Mature Stem Bark

Magnification of photographed drawing approximately 50x.

For description see page 24

# Plate z





### Plate VI

Sclerenchyma Elements Isolated by Schulze's

Maceration Process

Magnification of photographed drawing approximately 100x.

For description see pages 24-25

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